Supplemental Figure 1. Evolutionarily conserved Boymaw ORF3 in primates. (a) Conservation of exon-intron splicing junctions (GT-AG) of the Boymaw gene in primates. **(b)** The putative protein encoded by the ORF3 displays high homology except a few amino acid substitutions in primates. **(c)** Immunocytochemical staining of the HA tagged proteins in COS-7 cells transfected with the four different constructs. In contrast to abundant expression in numerous cells expressing the Boymaw-HA-FT-mut gene, only a few cells displayed weak expression from the transfection of the Boymaw-HA plasmids.

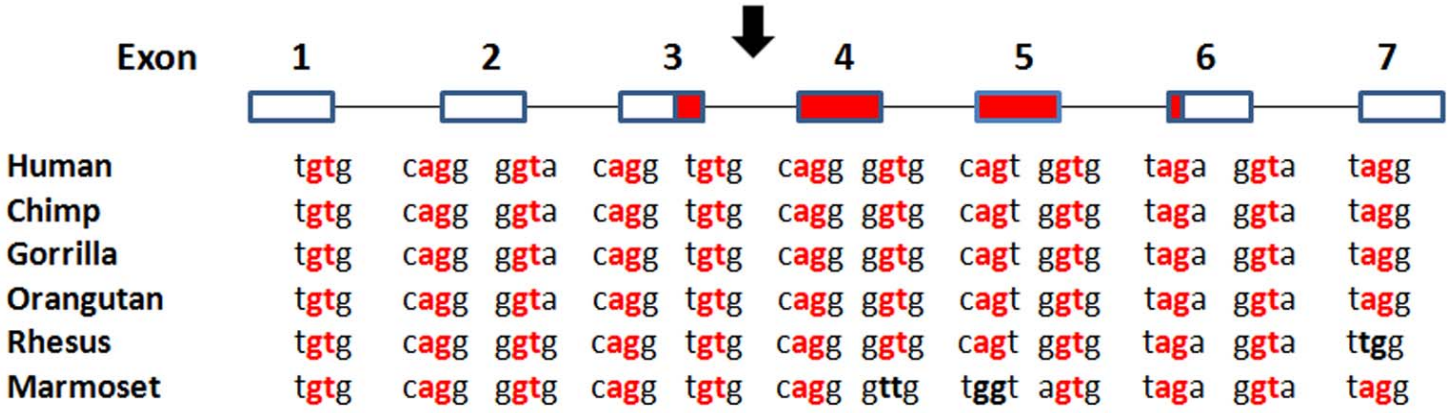
Supplemental Figure 2. Boymaw ORF3 protein localization and cell proliferation. **(a)** 15 consecutive optical sections of images from confocal microscopy were superimposed to create a 3D view of cells expressing the full-length Boymaw ORF3 proteins. (Blue: DAPI, red: Cytochrome C, green: Boymaw) **(b)** HEK293T cells were seeded at cell density 1 X 10^5 cells per well 20 h prior transfection. 48 h after transfection, cell numbers were counted. There were 6 replica wells for each construct. There was no difference in cell proliferation between cells transfected with Boymaw-HA-FT, Boymaw-HA-ACG, Boymaw-HA, and Boymaw-HA-mut constructs ($F(3,20)=0.23$, n.s.) . Decreased MTT reduction, rRNA expression, and protein translation in cells expressing either the Boymaw-HA or the Boymaw-HA-mut genes, therefore, was not caused by decreased cell numbers.

Supplemental Figure 3. Hypoxia has little effect on translation of the Boymaw ORF3. (a) Induction of HIF1α and Boymaw RNA expression with different concentration of $CoCl_2$. **(b)** Hypoxia has no effect on the translation of the Boymaw ORF3. The experiment was conducted in the same design as the starvation in Figure 4a.

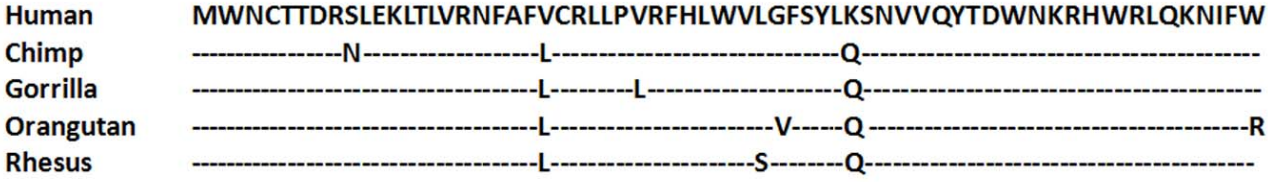
Supplemental Figure 4. Selection of primer pairs to obtain the same PCR amplification efficiency between the DISC1 and the Boymaw ORF3 genes. The following DISC1 and Boymaw primer pairs have the same amplification efficiency in SYBR Green real-time PCR amplification of their plasmid

templates: DISC1 (black): DISC1-F: GCAGCCAGCTCTTAGCAGTT; DISC1-R: CTCCATTCTCAGTGGGGTGT; Boymaw (red): Boymaw-F: AGCTTAAGACGTACTCCTCAAGGGG; Boymaw-R: CCTAAGACCCACAGATGGAAT.

a


Exon
1
2
3
4
5
6
7
Human tgtg cagg ggta cagg tgtg cagg ggtg cagt ggtg taga ggta tagg
Chimp tgtg cagg ggta cagg tgtg cagg ggtg cagt ggtg taga ggta tagg
Gorrilla tgtg cagg ggta cagg tgtg cagg ggtg cagt ggtg taga ggta tagg
Orangutan tgtg cagg ggta cagg tgtg cagg ggtg cagt ggtg taga ggta tagg
Rhesus tgtg cagg ggtg cagg tgtg cagg ggtg cagt ggtg taga ggta ttgg
Marmoset tgtg cagg ggtg cagg tgtg cagg gttg tggt agtg taga ggta tagg

b


Human MWNCTTDRSLEKLTLVRNFAFVCRLLPVRFHLWVLGFSYLKSNVVQYTDWNKRHWRLQKNIFW
Chimp ----------N----------L----------Q----------
Gorrilla ----------L--------L----------Q----------
Orangutan ----------L----------V-----Q----------R
Rhesus ----------L----------S-------Q----------

c

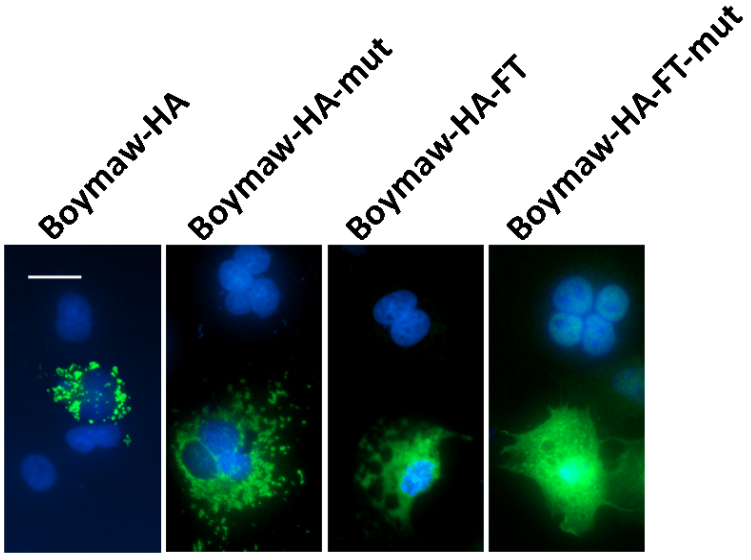
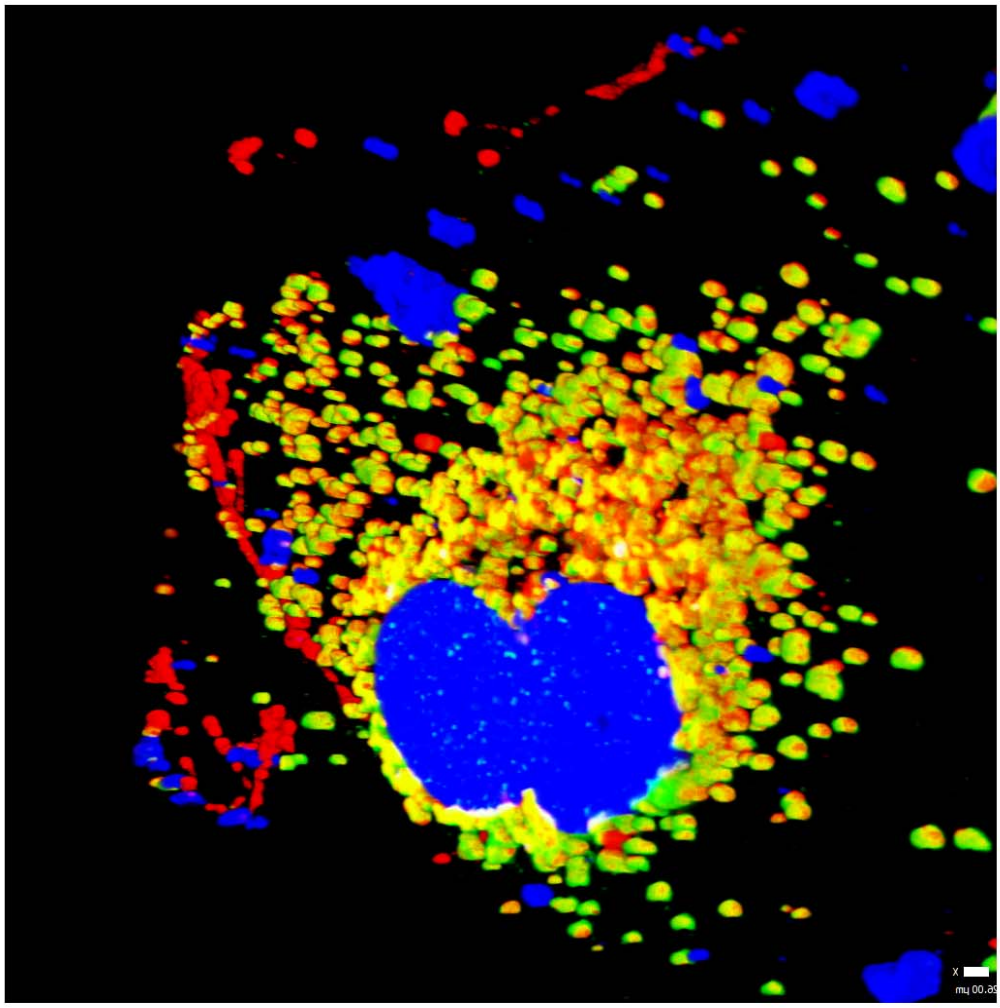

Boymaw-HA
Boymaw-HA-mut
Boymaw-HA-FT
Boymaw-HA-FT-mut

Fig S1

a



b

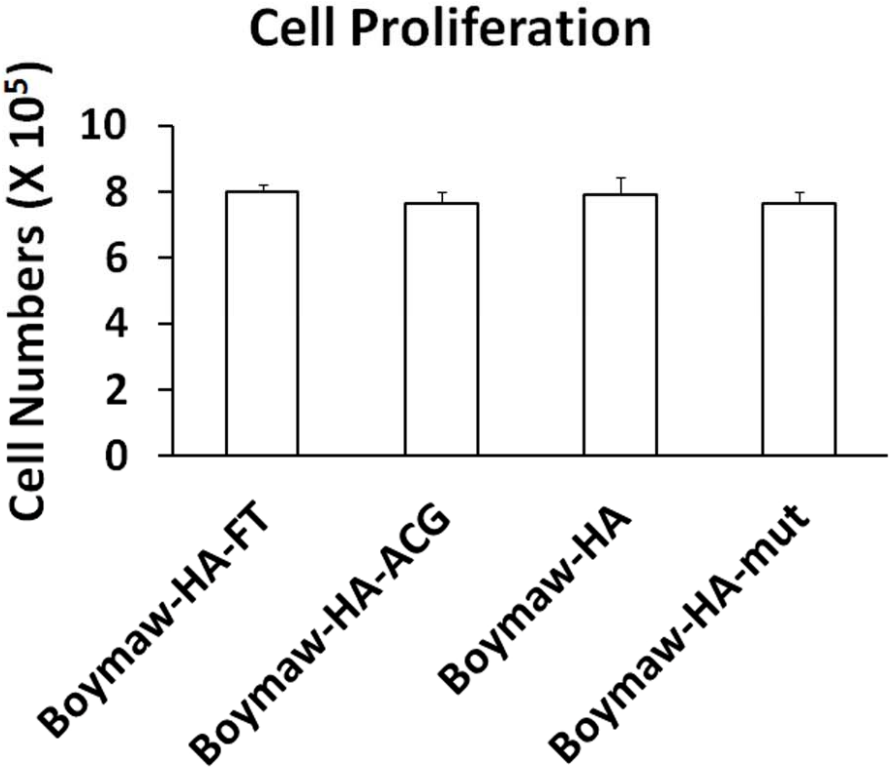
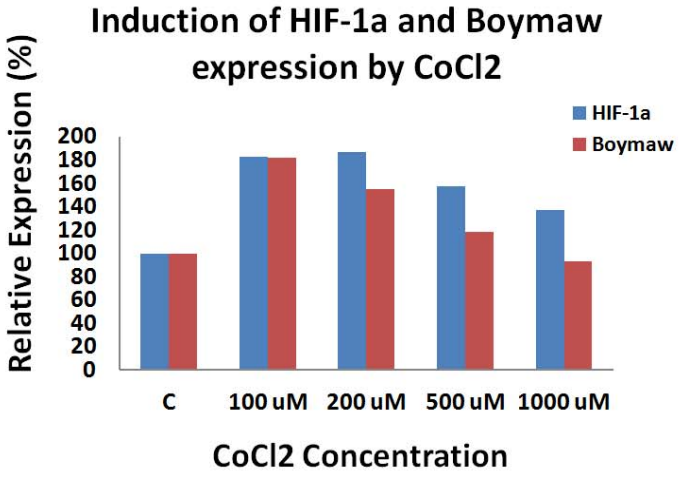

Cell Proliferation
Cell Numbers (X 10^5)
10
8
6
4
2
0
Boymaw-HA-FT
Boymaw-HA-ACG
Boymaw-HA
Boymaw-HA-mut

Fig S2

a


Induction of HIF-1a and Boymaw expression by CoCl2
Relative Expression (%)
200
180
160
140
120
100
80
60
40
20
0
HIF-1a
Boymaw
C
100 uM
200 uM
500 uM
1000 uM
CoCl2 Concentration

b

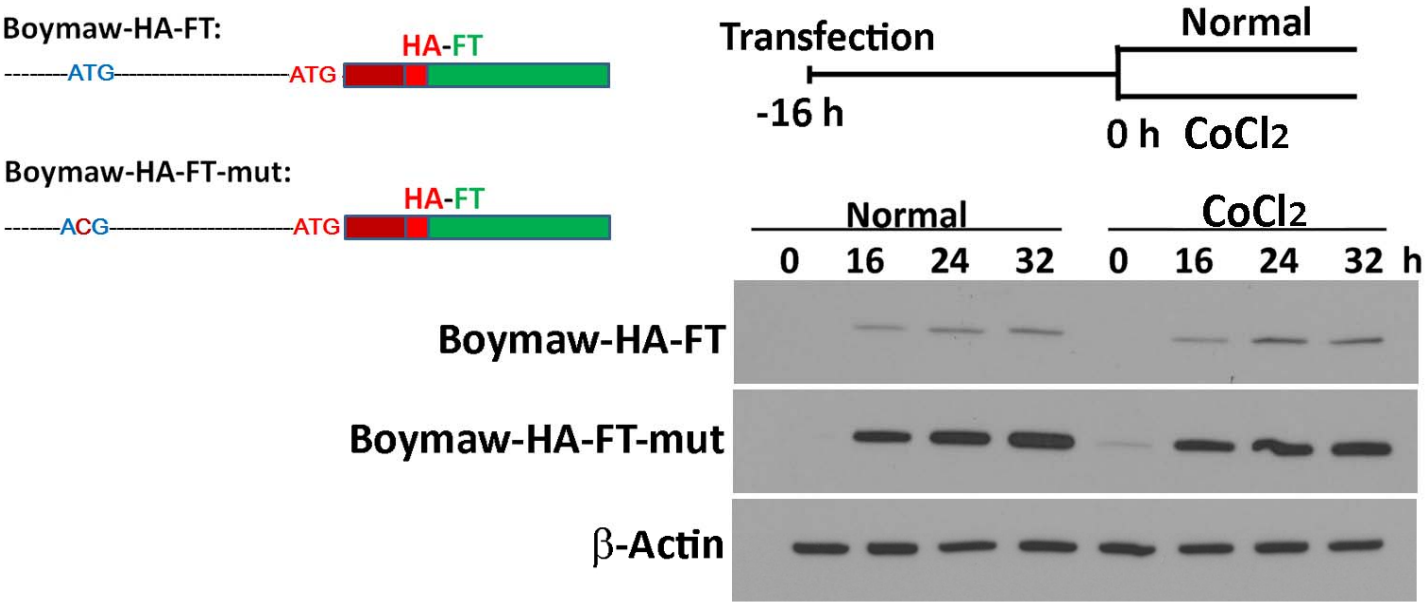

Boymaw-HA-FT:
-------ATG----------ATG
HA-FT
Boymaw-HA-FT-mut:
-------ACG----------ATG
HA-FT
Transfection
Normal
-16 h
0 h
CoCl2
Normal
CoCl2
0 16 24 32 0 16 24 32 h
Boymaw-HA-FT
Boymaw-HA-FT-mut
β-Actin

Fig S3

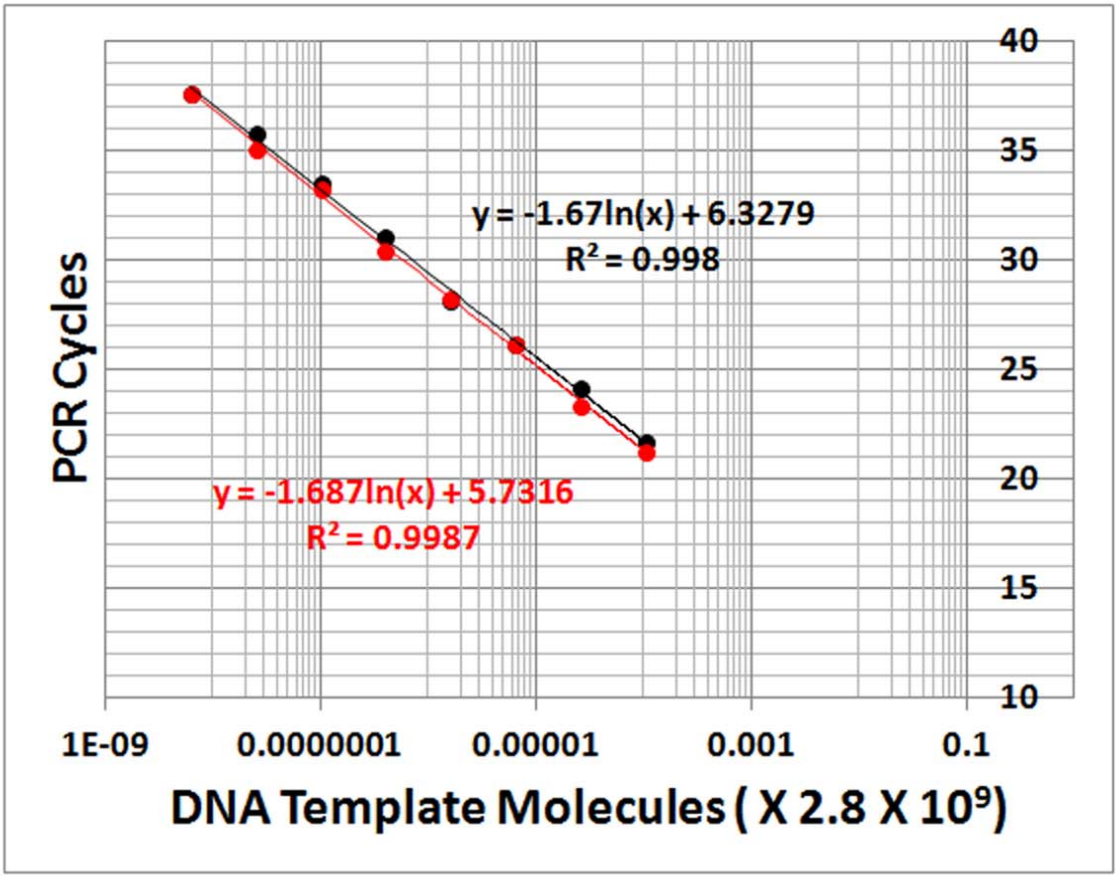

PCR Cycles
40
35
30
25
20
15
10
y = -1.67ln(x) + 6.3279
R² = 0.998
y = -1.687ln(x) + 5.7316
R² = 0.9987
1E-09
0.0000001
0.00001
0.001
0.1
DNA Template Molecules (X 2.8 X 10⁹)

Fig S4